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Seasonal characteristics of gut bacterial communities associated with carpenter ant *Camponotus japonicus* (Hymenoptera: Formicidae)

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Abstract: 【Aim】Ants have evolved a complicated association with microbes and, in particular, gut bacteria may have a strong impact on dietary evolution and species diversification of ants. Ants of the genus *Camponotus* are well known for harboring the endosymbiont *Blochmannia* and some other bacteria, which play an important role in the supplement of host nutrition. However, gut bacterial communities are sensitive to change of the food type, and such information might be useful to investigate the feeding activity of host ants in different seasons. In this study we aim to reveal whether the gut bacterial communities present seasonal characteristics in *Camponotus* ants. 【Methods】The gut bacterial communities of foraging workers of *C. japonicus* collected from two colonies (colony 1 and 2) on four sampling dates (June 12, August 15, and October 10 in 2012, April 15 in 2013) were investigated using the 16S rRNA polymerase chain restriction fragment-length polymorphism (PCR-RFLP) method. 【Results】Seventeen bacterial genera and one unknown bacterium were found, dominated by *Blochmannia* in all the eight samples (67.1% -98.8%). Bacteria of *Pseudomonas* and *Enterobacter* were present in most samples, but other bacteria were distributed only sporadically in one to three samples with very low clone numbers. The gut bacterial communities in the two colonies did not show consistent change trend in the four months, and that they all harbored a low bacterial diversity. In colony 1, the bacterial diversity was relatively higher in April and October, but much lower in June and August, while in colony 2, the bacterial diversity was much higher in August than in April, June and October. The two colonies had similar bacterial communities in June and October, but were distinctly different in August and April. 【Conclusion】The gut bacterial composition and diversity in two colonies of *C. japonicus* can shift with seasons, but such changes have no consistent trend and no obviously season-specific characteristics have been acquired by this ant.

Key words: *Camponotus japonicus*; gut bacteria; 16S rRNA-RFLP; seasonal characteristics; bacterial diversity

1 INTRODUCTION

Ants are dominant creatures in terrestrial ecosystems due to their species richness and extraordinary abundance (Hölldobler and Wilson, 1990), and they have evolved a complicated association with microbes (Boursaux-Eude and Gross, 2000; Zientz *et al*., 2005; Funaro *et al*., 2011; Seipke *et al*., 2013; Engel and Moran, 2013; Witek *et al*., 2014). In particular, gut bacteria have had a strong impact on dietary evolution and species diversification of ants. For example, gut bacteria support herbivorous ants to overcome their dietary deficiencies (Boursaux-Eude and Gross, 2000; van Borm *et al*., 2002; Russell *et al*., 2009; Anderson *et al*., 2012). The obligate endosymbionts *Blochmannia* have been found in the midgut and ovaries of all known species of *Camponotus* ants (Sauer *et al*., 2000), which can supplement host nutrition through a combination of nitrogen recycling and upgrading of nonessential amino acids (Sauer *et al*., 2002; Feldhaar *et al*., 2007; Williams and Wernegreen, 2010). Recent studies revealed that guts of two *Camponotus* species harbor other bacteria besides *Blochmannia* (He *et al*., 2011; Li *et al*., 2012; He *et al*., 2014).

Camponotus ants are generally considered to be omnivorous and have been recorded as feeding on a wide variety of food, including honeydew, insect preys, plant nectar, and fruit juices (Wu and Wang, 1995; Hansen and Klotz, 2005). However, due to the filtration of an infrabuccal pocket in their

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preoral cavity (Eisner and Happ, 1962; Hansen *et al*., 1999), they actually only utilize liquid food resources (Alsina *et al*., 1988; Cannon and Fell, 2002). Foraging workers gather and store food for the whole colony, and food types collected by foraging workers can usually be examined through field investigation, direct measurements of crop contents, stable isotopes, *etc*. (Cannon and Fell, 2002; Davidson *et al*., 2003; Blüthgen *et al*., 2003). However, these methods usually need more time and workloads, and it is still difficult to discover what nutritions ants actually utilize and how they are distributed in their nests.

It was reported that foraging activities of *Camponotus* ants had a clear seasonal pattern for nutritional demand at different developmental stages (Rico-Gray and Sternberg, 1991), which implies that foraging workers can modulate their food collection activity according to the nutritional requirements of the colony.

Gut bacterial communities have developed a close relationship with host animals, and they were usually affected by many factors, such as gut structure and physiology, as well as diet and host living environment (Brune and Friedrich, 2000; Russell *et al*., 2009; He *et al*., 2014), especially diet change caused by season shift is an important factor that influences gut microflora of animals (Pinto-Tomás *et al*., 2011; Colman *et al*., 2012; Davenport *et al*., 2014; Zhang *et al*., 2014; Amato *et al*., 2015). Ants are social insects that usually have relatively immobile nests, and they have to continuously forage outside their nests from spring to autumn, since their diets should be closely related to the food resource around their living environment. But it is still unknown if the gut bacteria of foraging workers could change with the season shift, and if gut bacteria can reflect their modulating ability of food collection according to the nutritional requirements of the colony. The carpenter ant *Camponotus japonicus* is widely distributed in China, which is a good natural enemy for many forest pests (Wang *et al*., 1991; Wu and Wang, 1995). Like other *Camponotus* ants, *C. japonicus* feeds on honeydew, insect preys, plant nectar and fruit juices during its forage activity from spring to autumn. Our recent researches revealed that the digestive tracts of *C. japonicus* harbor other bacteria apart from the endosymbionts *Blochmannia*, *e. g*., *Candidatus* Serratia symbiotica, *Fructobacillus fructosus*, *Burkholderiales*, *Pseudomonas*, *Bacillus*, *Enterococcus*, and *Paenibacillus* (He *et al*., 2011; Li *et al*., 2012; Zhu *et al*., 2015). In this study, we collected foraging workers of *C. japonicus* in different months (April, June, August, and October) and analyzed their bacterial communities in digestive tracts by using 16S rRNA-RFLP method, aiming to reveal the gut bacterial composition of foraging workers in different seasons and the relationship between gut bacteria and their seasonal feeding activity.

2 MATERIALS AND METHODS

2.1 Ant collection

Two mature colonies of *C. japonicus*, 3 000 m apart from each other, located in Yangling, Shaanxi Province of China, were investigated in this study. The first colony (colony 1) located in the rhizosphere of *Populus tomentosa* (roadside shade trees about more than 60 years old), no vegetation was distributed around the tree stem and there were other urban green plants nearby about 2 meters, and this colony was affected by human activity such as trampling and watering. The second colony (colony 2) was located in *Platycladus orientalis* woods (about 20 years old) on the campus of Northwest A&F University, and there were lots of other green plants around, but sparse herbaceous plants on the woods ground, with relatively less human impact. Workers in both colonies forage on the ground or climb on the trees feeding on aphid honeydew. In this area climate presents obvious seasonal change, and foraging workers start foraging activity from April through to the end of October. Foraging workers were collected at the same day from the two colonies on four sampling dates, and for each colony, more than 15 worker individuals were carefully collected with forceps and put separately into respective centrifugal tubes, and then all tubes were placed into an insulated cooler to make them inactive and brought back to the laboratory for dissection as soon as possible. Samples were collected on June 12, August 15, and October 10 in 2012, and April 15 in 2013, respectively. The average temperature and precipitation were as follows: 25.2℃ (14.4 – 37℃) and 30 mm in June, 24.1℃ (15.3 – 34.9℃) and 152.3 mm in August, 13℃ (8 – 24℃) and 40 – 50 mm in October, and 15°C (1.3 – 32℃) and 13.2 mm in April, respectively. All data of temperature and precipitation were from the meteorological monthly reports of Weather Bureau in Yangling, Shaanxi Province. Sample numbers were given according to the collecting time (month) and ant colony, *i. e*., sample 4C1 representing a sample collected from colony 1 on April 15, 2013; 4C2 representing a sample collected from colony 2 on

April 15, 2013; 6C1 representing a sample collected from colony 1 on June 12, 2012; 6C2 representing a sample collected from colony 2 on June 12, 2012; 8C1 representing a sample collected from colony 1 on August 15, 2012; 8C2 representing a sample collected from colony 2 on August 15, 2012; 10C1 representing a sample collected from colony 1 on October 10, 2012; 10C2 representing a sample collected from colony 2 on October 10, 2012.

2.2 Dissection of ant guts and DNA extraction

The worker was first anesthetized in −20℃ freezer for 5 − 10 min, then externally sterilized with 70% ethanol for 1 min, and washed twice with sterilized water. After that, worker was fixed on a wax plate with abdomen immersed in sterile 0.9% NaCl solution, the abdominal cuticula were rived and cleaned out by using sterilized fine-tip forceps. The whole gut was washed twice with sterile 0.9% NaCl solution immediately after exposed. The hemolymph and NaCl solution around the gut were absorbed with filter paper, and the gut was carefully put into microcentrifuge tubes (1.5 mL). If the gut was broken before removal, it would be discarded. The dissection work was performed in the laminar flow cabinet according to the method previously reported (Li *et al*., 2012). Genomic DNA of gut tissues was extracted from pooled digestive tracts of 15 workers for each sample, and a total of eight samples were extracted. The DNA extraction was done using the Genome DNA Extraction Kit (Tiangen Inc., Beijing) according to the manufacturer's instructions.

2.3 PCR amplification, cloning, and restriction fragment-length polymorphism analysis

The 16S rRNA genes were amplified with universal bacterial primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TAGGGYTACCTTGTTACGACTT-3′) (Broderick *et al*., 2004). PCR was performed using a Peltier Thermal Cycler (Eppendorf Mastercycler proS, Germany). The PCR reaction mixtures (50 μL) contained 5 μL of 10 × PCR buffer, 3 μL of $MgCl_2$ (25 mmol/L), 4 μL of dNTPs Mixture (2.5 mmol/L), 2 μL of each primer (10 mmol/L), 0.5 μL of Taq DNA polymerase (5 U/mL; TaKaRa Bio Inc.), 4 μL template DNA, and 29.5 μL sterile ddH_2O. The amplification program consisted of an initial denaturation step at 94℃ for 2 min, then followed by 28 cycles of annealing (94℃ 30 s, 55℃ 45 s, and 72℃ 60 s), and a final extension step (72℃, 10 min). The PCR product was approximately 1 500 bp, it was purified with a TaKaRa Agarose Gel DNA Purification Kit (TaKaRa Bio Inc.) according to the manufacturer's instructions, and then it was ligated into the pMD19-T vector (TaKaRa Bio Inc.) and transformed into *Escherichia coli* cells (DH5α) by heat-shock. The transformed *E. coli* cells containing M13 plasmid with ligated 16S rRNA inserts were spread onto LB agar plates amended with 20 mg/mL X-Gal, 20 mg/mL IPTG and 100 mg/mL ampicillin, and then incubated at 37℃ for 12 − 18 h. A total of 500 white clones were picked in a random order with sterile toothpicks and subcultured onto 1 cm × 1 cm grids (drawn and numbered on the bottom of the plates in advance) in new LB agar plates with 100 mg/mL ampicillin. After 24-h incubation, 200 white clones were selected to amplify with universal M13 vector primers to check if they were positive clones. The positive clones were further analyzed by restriction fragment-length polymorphism (RFLP) analysis. Eight clone libraries of 16S rRNA gene were constructed.

PCR products from positive clones using M13 vector primer were digested separately with *Afa*I and *Hha*I restriction endonucleases according to the manufacturer's instructions. Digested products were separated on 2% agarose gel electrophoresis in 1 × TAE buffer, and then the gels were stained with ethidium bromide and visualized under UV light. The RFLPs of all positive clones were compared and visually grouped, and different RFLPs were calculated. One to three representative clones for each unique RFLP were sequenced in both forward and reverse directions using 16S rRNA primers (27F and 1492R) at the Shanghai Sangon Biological Engineering Technology and Service Co., Ltd. in China.

2.4 Data analysis

Sequences were assembled and manually corrected using Chromas Lite 2.01 (Technelysium Pty Ltd, Australia). In order to determine the correct taxonomical affiliation of each sequence, all sequences were blasted in both GenBank and Ribosomal Database Project (http://rdp.cme.nsu.edu/) to search their closest matched relatives. All sequences we obtained and their closest matched sequences were aligned using Clustal X 2.1 (Larkin *et al*., 2007), and aligned sequences were loaded to construct a Maximum Likelihood tree in MEGA 5 (Tamura *et al*., 2011). Taxonomic descriptions were determined based on the position of each aligned sequence in the phylogenetic tree. Operational taxonomic units (OTUs) were defined at a 97% nucleotide similarity. Diversity indices and clone coverage (C) were calculated using SPADE software (http://chao. stat. nthu. edu. tw/blog/software-download/spade/) to evaluate the bacterial diversity and sampling number of clone library. Rarefaction curves were used to gauge the adequacy of sampling with Analytic Rarefaction 1. 3 software (UGA Stratigraphy Lab, http://strata. uga. edu/software/anRareReadme. html). Multidimensional scaling (MDS) analysis was used to present the space expression of the similarity among samples with the program SPSS 19. 0 for Windows.

All nucleotide sequences were deposited in the NCBI/Blast/GenBank nucleotide sequence databases under the following accession numbers: KJ424414 – KJ424432.

3 RESULTS

3.1 Composition of the gut bacteria of *C. japonicus*

For each clone library, PCR products of about 200 positive clones were digested with *Afa* I and *Hha* I restriction endonucleases, and the different RFLPs were counted. There were 46, 22, 20, 16, 12, 9, 15, and 7 RFLPs in the eight 16S rRNA gene libraries (8C2, 10C1, 4C1, 10C2, 6C1, 6C2, 8C1, and 4C1), respectively. The representative clones for each unique RFLP were sequenced in each clone library.

Based on the sequence blasting results of the representative clones (Table 1), 17 bacterial genera and one unknown bacterium were determined in the eight 16S rRNA gene libraries associated with *C. japonicus*. They belong to five phyla, *i. e.*, Proteobacteria, Firmicutes, Actinobacteria, Deinococcus-Thermus and Bacteroidetes (Fig. 1). *Blochmannia* was the most frequently identified bacterium in each library, accounting for 77. 6% in 4C1, 96. 2% in 4C2, 95. 2% in 6C1, 98. 8% in 6C2, 97. 8% in 8C1, 67. 1% in 8C2, 88. 8% in 10C1, and 87. 2% in 10C2 (Table 1), respectively. *Psedomonas* and *Enterobacter* were the other two common groups found in six and five libraries, respectively (Table 1). Other bacteria only presented sporadically in one to three libraries with very low clone numbers (Table 1).

Table 1 Bacterial taxa associated with gut samples of *Camponotus japonicus* collected from Yangling, Shaanxi in 2012 – 2013

Identification from closest match in GenBank	Number of clones							
	4C1	4C2	6C1	6C2	8C1	8C2	10C1	10C2
Proteobacteria								
Blochmannia japonicus	128	179	158	164	182	145	174	171
Pseudomonas sp.	20	6	/	2	/	30	7	8
Enterobacter sp.	1	1	1	/	4	3	/	16
Uncultured bacterium	4	/	/	/	/	/	/	/
Acinetobacter sp.	7	/	/	/	/	1	/	/
Serratia sp.	/	/	1	/	/	2	/	/
Saccharibacter floricola	1	/	/	/	/	/	/	/
Burkholderia sp.	/	/	7	/	/	2	1	/
Rhizobium sp.	1	/	/	/	/	/	1	/
Xenorhabdus sp.	/	/	/	/	/	/	3	/
Firmicutes								
Fructobacillus fructosus	/	/	/	/	/	17	/	/
Lactococcus lactis	/	/	/	/	/	13	/	/
Actinobacteria								
Rhodococcus sp.	/	/	/	/	/	/	3	1
Agrococcus sp.	/	/	/	/	/	/	2	/
Deinococcus-Thermus								
Meiothermus cerbereus	3	/	/	/	/	2	1	/
Bacteroidetes								
Flavobacterium sp.	/	/	/	/	/	/	1	/
Pedobacter sp.	/	/	/	/	/	/	1	/
Sphingobacterium sp.	/	/	/	/	/	1	2	/
Total clone number	165	186	166	166	186	216	196	196

4C1: First ant colony collected on April 15, 2013; 4C2: Second ant colony collected on April 15, 2013; 6C1: First ant colony collected on June 12, 2012; 6C2: Second ant colony collected on June 12, 2012; 8C1: First ant colony collected on August 15, 2012; 8C2: Second ant colony collected on August 15, 2012; 10C1: First ant colony collected on October 10, 2012; 10C2: Second ant colony collected on October 10, 2012. /: Undetected in the sample.

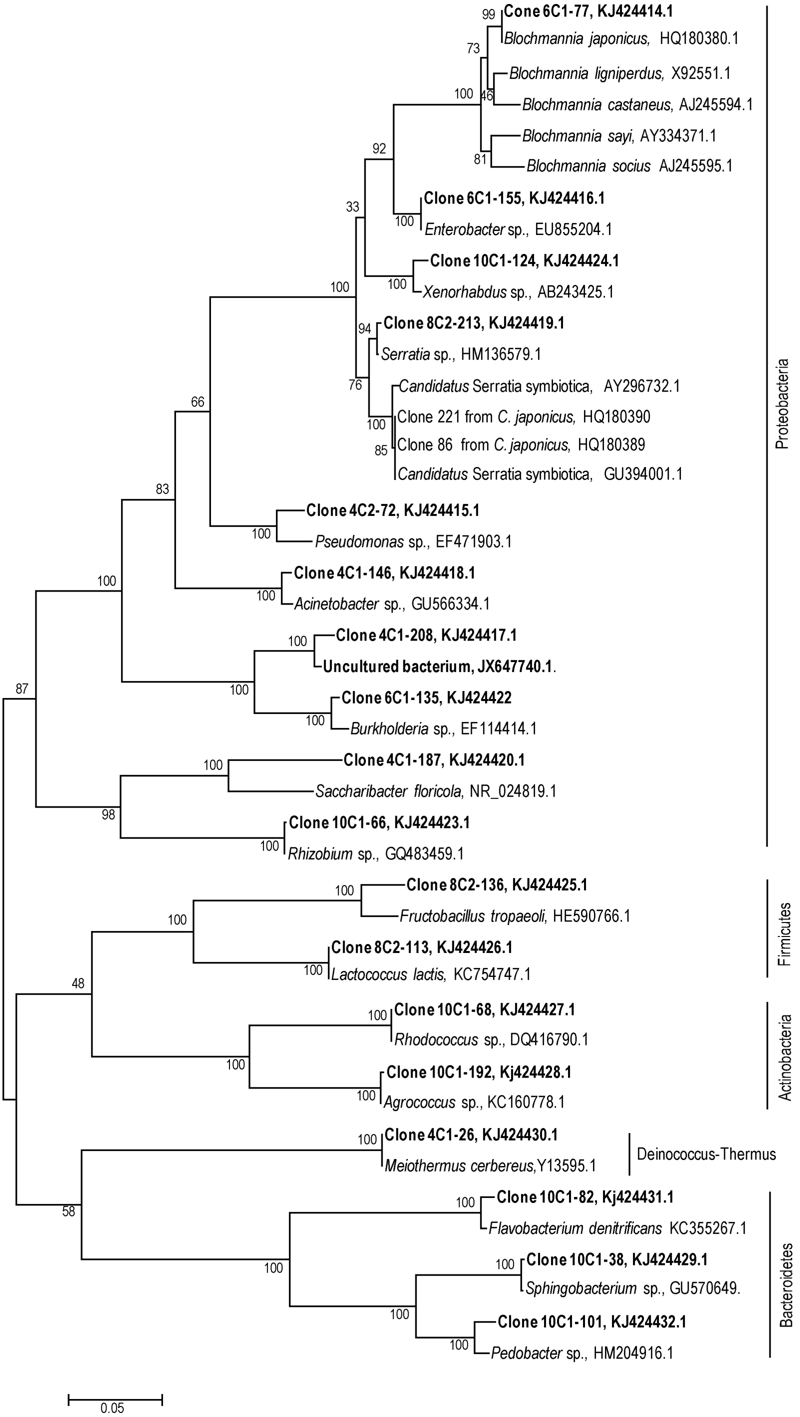
99
Cone 6C1-77, KJ424414.1
73
Blochmannia japonicus, HQ180380.1
Blochmannia ligniperdus, X92551.1
100
46
Blochmannia castaneus, AJ245594.1
Blochmannia sayi, AY334371.1
92
81
Blochmannia socius AJ245595.1
33
Clone 6C1-155, KJ424416.1
100
Enterobacter sp., EU855204.1
Clone 10C1-124, KJ424424.1
100
100
Xenorhabdus sp., AB243425.1
94
Clone 8C2-213, KJ424419.1
Serratia sp., HM136579.1
76
Candidatus Serratia symbiotica, AY296732.1
66
100
Clone 221 from C. japonicus, HQ180390
85
Clone 86 from C. japonicus, HQ180389
Candidatus Serratia symbiotica, GU394001.1
83
Clone 4C2-72, KJ424415.1
100
Pseudomonas sp., EF471903.1
100
Clone 4C1-146, KJ424418.1
100
Acinetobacter sp., GU566334.1
100
Clone 4C1-208, KJ424417.1
Uncultured bacterium, JX647740.1.
87
100
Clone 6C1-135, KJ424422
100
Burkholderia sp., EF114414.1
100
Clone 4C1-187, KJ424420.1
Saccharibacter floricola, NR_024819.1
98
Clone 10C1-66, KJ424423.1
100
Rhizobium sp., GQ483459.1
100
Clone 8C2-136, KJ424425.1
100
Fructobacillus tropaeoli, HE590766.1
Clone 8C2-113, KJ424426.1
48
100
Lactococcus lactis, KC754747.1
100
Clone 10C1-68, KJ424427.1
Rhodococcus sp., DQ416790.1
100
Clone 10C1-192, Kj424428.1
100
Agrococcus sp., KC160778.1
100
Clone 4C1-26, KJ424430.1
Meiothermus cerbereus,Y13595.1
100
Clone 10C1-82, Kj424431.1
58
Flavobacterium denitrificans KC355267.1
100
100
Clone 10C1-38, KJ424429.1
Sphingobacterium sp., GU570649.
100
Clone 10C1-101, KJ424432.1
100
Pedobacter sp., HM204916.1
Proteobacteria
Firmicutes
Actinobacteria
Deinococcus-Thermus
Bacteroidetes
0.05

Fig. 1 ML Phylogenetic tree based on 16S rRNA sequences of representative clones belonging to the gut bacteria of *Camponotus japonicus* and their closest matched sequences downloaded from GenBank

The tree was generated using the Maximum Likelihood method with 2 000 bootstraps and General Time Reversible model in MEGA5 software. The scale bar represents 0.05 substitutions per nucleotide position. All the sequences obtained in this research are listed in boldface type followed by their GenBank accession numbers.

3.2 Diversity and similarity of gut bacteria of *C. japonicus*

Analyses of richness and composition of bacterial species (Table 2) presented that the gut bacterial communities in the two colonies did not show consistent change trend in the four months, and that they all harbored a low bacterial diversity. In colony 1, the bacterial diversity was relatively higher in April and October, but much lower in June and August, while in colony 2, the bacterial diversity was much higher in August than in April, June and October. Among the eight samples, 8, 10, and 11 bacterial genera were detected from 4C1, 8C2, and 10C1, respectively, which showed a relatively high diversity index (Shannon index are 0.747, 1.150 and 0.581, respectively). The bacterial diversity index in other samples, however, was relatively very low (Shannon index *H*, 0.065 – 0.481). The Simpson index within samples was higher than Shannon index except for 4C1 and 8C2, implying that dominant bacteria play an important role in gut bacterial communities in *C. japonicus*.

Multi-dimensional scaling (MDS) analysis of gut bacterial community structure showed close similarities among the samples 4C2, 8C1, 10C1, 10C2, 6C1 and 6C2, except for 8C2 and 4C1 (Fig. 2). The two colonies had similar bacterial communities in June (6C1 and 6C2) and October (10C1 and 10C2), but were distinctly different in August (8C1 and 8C2) and April (4C1 and 4C2).

The clone coverage (C) values of the eight clone libraries were all higher than 0.9 (Table 2), and the rarefaction curves for them reached plateaus at 3% difference between sequences (95% confidence) (Fig. 3). These suggest that the number of sampling clones was sufficient to provide an accurate estimation of gut bacterial diversity in all the eight clone libraries.

4 DISCUSSION

4.1 Gut bacterial communities of *C. japonicus*

Gut bacterial communities in the two colonies of *C. japonicus* investigated in the present study showed inconsistent seasonal changes. Among the identified bacteria, the endosymbionts *Blochmannia* was the most dominant bacterium, which accounted for 67.1% – 98.8% in all the eight samples. Although other bacterial groups were also present, they occurred

Table 2 Diversity indices of 16S rRNA gene libraries calculated from eight samples (95% confidence interval) of *Camponotus japonicus* collected from Yangling, Shaanxi in 2012 – 2013

Samples	Total number of clones (N)	Species richness	Shannon index	Simpson index	Clone coverage (C)
4C1	165	8	0.747	0.647	0.981
4C2	186	3	0.176	0.927	0.995
6C1	166	3	0.211	0.907	0.994
6C2	166	2	0.065	0.976	1.000
8C1	186	2	0.104	0.958	1.000
8C2	216	10	1.150	0.478	0.991
10C1	196	11	0.581	0.789	0.974
10C2	196	4	0.481	0.768	0.995

4C1: First ant colony collected on April 15, 2013; 4C2: Second ant colony collected on April 15, 2013; 6C1: First ant colony collected on June 12, 2012; 6C2: Second ant colony collected on June 12, 2012; 8C1: First ant colony collected on August 15, 2012; 8C2: Second ant colony collected on August 15, 2012; 10C1: First ant colony collected on October 10, 2012; 10C2: Second ant colony collected on October 10, 2012.

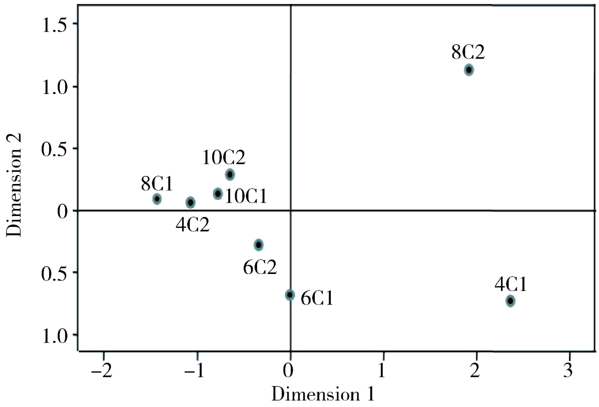

Fig. 2 Two dimensional MDS map of bacterial community composition of eight samples

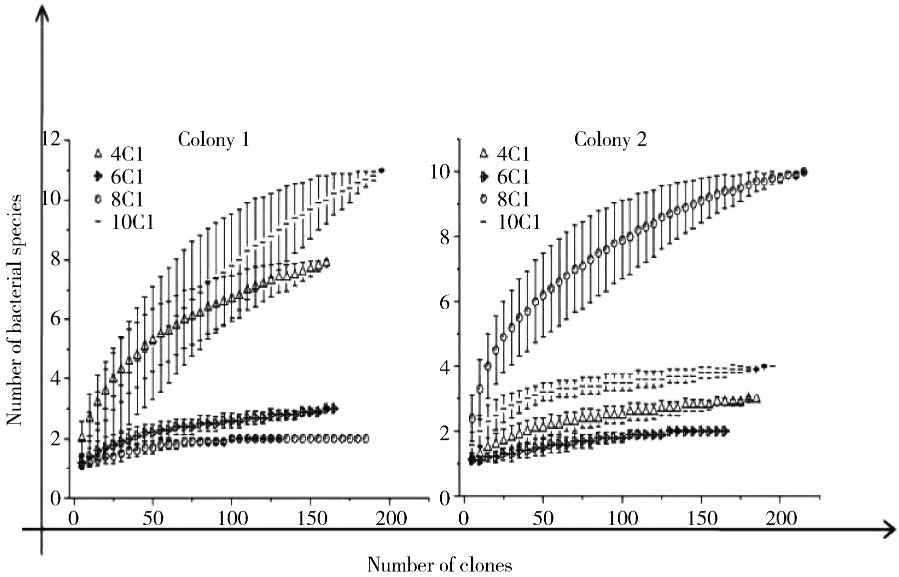

Fig. 3 Rarefaction curves for 16S rRNA gene libraries constructed from eight samples

Error bars represent 95% confidence interval for richness values.

much sporadically, with low clone numbers in several samples (Table 1). This finding is consistent with the results obtained by Feldhaar *et al.* (2007), He *et al.* (2011), and Li *et al.* (2012). But it is in stark contrast to the discovery reported in other ants, such as herbivorous cephalotine ants whose gut harbored rich bacterial taxa (Russel *et al.*, 2009; Anderson *et al.*, 2012; Kautz *et al.*, 2013), and army ants which harbored abundant host-specific bacterial lineages (Funaro *et al.*, 2011).

Apart from *Blochmannia*, bacteria of *Pseudomonas*, *Enterobacter*, *Fructobacillus*, and *Serratia* were also detected in the guts of *C. japonicus* in this study, as shown in other related researches (He *et al.*, 2011; Li *et al.*, 2012; Zhu *et al.*, 2015). In particular, *Pseudomonas* and *Enterobacter* were found in most samples, suggesting that they could be the residential bacteria in guts of *C. japonicus*. He *et al.* (2011) detected *Candidatus* Serratia symbiotica, a secondary endosymbiont of aphids, from the gut of *C. japonicus*, and deduced that it was probably transferred horizontally from the cypress aphid *Cinara tujafilina* (del Guercio), as the foraging workers were often observed feeding on the honeydew of the aphids. *Serratia* has also been found from the midgut of another carpenter ant, *C. floridanus* (Feldhaar *et al.*, 2007). However, these bacteria were detected in only two samples here. This is probably due to that the secondary endosymbionts of aphids can not continuously exist in the honeydew (Baumann and Baumann, 2005).

4.2 Variation of gut bacteria of *C. japonicus* and its possible influencing factors

In this study, although the gut bacterial composition did not change in the two colonies in a consistent seasonal pattern, in each of them the bacterial communities did shift to a certain extent. The two colonies had similar bacterial communities in June (6C1 and 6C2) and October (10C1 and 10C2), but were distinctly different in August (8C1 and 8C2) and April (4C1 and 4C2) (Fig. 2), especially more bacterial species were isolated from three samples 4C1, 8C2 and 10C1 (Table 1). Such a deviation between the two colonies and different samples could be due to the micro-ecology environment of nest locations and/or the type of food they collected.

Moreover, the unique structure of digestive system may also influence the gut microflora of ants. In ants, an infrabuccal pocket in their preoral cavity can filter very small solid particles from food material and allow only semi-liquid to enter the midgut (Eisner *et al.*, 1962; Glancey *et al.*, 1981). Bacteria and yeasts associated with the infrabuccal pocket have been found in ants *C. modoc* (Wheeler) and *C. vicinus* (Mayr) (Hansen *et al.*, 1999; Mankowski and Morrell, 2004), and we also isolated many bacteria species from the pellets of infrabuccal pocket in the foraging workers of *C. japonicus* (Zhang *et al.*, 2016). It is unknown whether the infrabuccal pocket and its contents are an entirely different "habitat" for bacteria, but it appears to be certain that some bacteria were filtered into the infrabuccal pocket with food particles, and this structure may prevent harmful microbes entering the digestive tract.

In addition, our previous and present studies all showed that up to 98% of the clones found in guts of *C. japonicus* and field-collected workers of *C. fragilis* were *Blochmannia* (He *et al.*, 2011, 2014). This may be due to the large amount of *Blochmannia* harbouring in the gut tissue of *Camponotus* ants, and when performing a normal PCR using universal primers for the bacterial 16S rRNA gene other bacteria cannot be detected.

Moreover, in this study we still used 16S rRNA-RFLP method to analyze the gut bacteria of *C. japonicus* on basis of our previous researches, so as to further reveal if those previously isolated bacteria can be commonly found in the gut of *C. japonicus* and if their composition present seasonal change characteristic. The results showed that there was no obvious seasonal change in gut bacterial composition and diversity in these two colonies, which might be due to 16S rRNA PCR-RFLP is not a highly sensitive method to reveal bacterial diversity here. Thus in future we would try other more sensitive and effective methods like high-throughput sequencing to investigate the gut microflora of ants.

4.3 Conclusion

In conclusion, gut bacterial composition and diversity index in two colonies of *C. japonicus* can shift with seasons, but such changes do not have a consistent trend and no obviously season-specific characteristics have been acquired by this ant.

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日本弓背蚁肠道细菌群落的季节特征

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摘要:【目的】在长期的进化过程中,蚂蚁和微生物之间建立了复杂的联系,尤其肠道微生物对蚂蚁的食性进化和物种分化产生了巨大的影响。弓背蚁属 *Camponotus* 蚂蚁消化道内普遍存在内共生菌 *Blochmannia* 及其他肠道细菌,这些细菌在寄主蚂蚁营养补充方面发挥了重要的作用,此外肠道微生物对食物类型的变化十分敏感,这些信息可能有助于调查寄主蚂蚁在不同季节的取食习性。本研究旨在揭示弓背蚁属蚂蚁肠道微生物是否存在季节特征。【方法】采用 16S rRNA-RFLP 方法分析比较了了 2 个日本弓背蚁蚁巢(巢 1 和 2)的工蚁在 4 个月份时间点(2012 年 6 月 12 日,8 月 15 日和 10 月 10 日,2013 年 4 月 15 日)的肠道菌群组成。【结果】在 8 个样品中共发现了 17 个属的细菌和 1 种未知细菌,弓背蚁属蚂蚁特有的内共生菌 *Blochmannia* 是优势细菌,出现在所有样品中,占 67.1% ~98.8%;假单胞菌属 *Pseudomonas* 和肠杆菌属 *Enterobacter* 在大多数的样本中都检测到,其他属的细菌则零星分布在个别样品中,只占较低的比例。两个蚁巢工蚁肠道菌群在 4 个月份没有呈现一致的变化趋势,都具有低的细菌多样性。在巢 1 中,4 月和 10 月的肠道细菌多样性相对较高,在 6 月和 8 月较低;而在巢 2 中,8 月的肠道细菌多样性明显高于 4,6 和 10 月。两个蚁巢 6 月和 10 月的肠道菌群组成相似,但是 8 月和 4 月差异较大。【结论】日本弓背蚁两个蚁巢的工蚁肠道菌群组成和多样性都随季节产生变化,但是没有呈现一致的变化趋势,没有表现出明显的季节特征。

关键词: 日本弓背蚁; 肠道微生物; 16S rRNA-RFLP; 季节特征; 细菌多样性

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